A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

NeoBiota 102: 331–350 (2025)
DOI: 10.3897/neobiota.102.146951

Research Article

Eradication attempt for an early detected invasive crayfish: the case of *Pacifastacus leniusculus* (Decapoda, Astacidae) in the Clitunno River (central Italy)

Antonella Carosi[1], Francesca Lorenzoni[1], Fatemeh Zarei[1], Massimo Lorenzoni[1]

1 *Department of Chemistry, Biology and Biotechnologies, University of Perugia, via Elce di Sotto, 06123 Perugia, Italy*
Corresponding author: Antonella Carosi (antonella.carosi@unipg.it)

Abstract

The signal crayfish *Pacifastacus leniusculus* (Dana, 1852) is one of the most ecologically impactful decapod crustaceans introduced in Europe. As a species of Union concern, early detection and rapid eradication measures are required to prevent its establishment and spread. We aimed to: i) test the effectiveness of an eradication attempt undertaken to counteract the spread and contain the demographic growth of *P. leniusculus* in the Clitunno River Basin (central Italy), where the species has been detected in 2020; ii) assess distribution, age structure and growth of *P. leniusculus* in this invaded area. The removal actions were conducted biweekly, from June 2022 to December 2024, using both traps and electrofishing. Biometric parameters were individually recorded, and the demographic features and growth of *P. leniusculus* population were assessed. In total, 259 removal activities were carried out and a biomass of 39.74 kg was removed. The presence of six cohorts (from 0+ to 5+), including the young-of-the-year (0+), attested to the rapid acclimatisation of *P. leniusculus*, which gave rise to a self-sustaining population in a short time. The greatest removal efforts have been concentrated on a small tributary, named Fosso Vecchio, where the average values of Catch Per Unit Effort (CPUE) showed a significant decreasing trend over time, suggesting the effectiveness of the removal actions. Our findings provided some evidence that early detection and eradication measures conducted on a small scale, but with high effort, represent effective management tools to limit population abundance and prevent invasive crayfish from spreading further.

Key words: Biodiversity conservation, chalk streams, inland waters, invasive crayfish, removal activities

OPEN ACCESS

Academic editor: Pedro Anastácio
Received: 16 January 2025
Accepted: 12 June 2025
Published: 7 October 2025

Citation: Carosi A, Lorenzoni F, Zarei F, Lorenzoni M (2025) Eradication attempt for an early detected invasive crayfish: the case of *Pacifastacus leniusculus* (Decapoda, Astacidae) in the Clitunno River (central Italy). In: Anastácio P, Ribeiro F, Chainho P (Eds) Invasions in Aquatic Systems. NeoBiota 102: 331–350. https://doi.org/10.3897/neobiota.102.146951

Copyright: © Antonella Carosi et al.
This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Introduction

Biological invasions represent one of the major causes of biodiversity loss in freshwaters (Ricciardi and MacIsaac 2011; Reid et al. 2019). Acting as powerful generalist omnivores, crayfish are included amongst the most successful invaders in these environments (Gherardi 2012; Twardochleb et al. 2013; Wacker and Harzsch 2021; Soto et al. 2023; Carvalho et al. 2025). Crayfish have strong opportunities for invading aquatic systems due to their capacity to enter the food chain at various trophic levels and to exploit the considerable energy reserves of the detrital pool (Gherardi 2007). In the invaded areas, significant management efforts are required to prevent damage from alien crayfish, since they have the potential to directly affect native species (through predation, competition for food and shelters, disease transmission, etc.) as well as indirectly

affect ecosystems (through food webs and habitat alterations) (Ficetola et al. 2012; Twardochleb et al. 2013). North American crayfish are of particular concern, considering their ability to assume a preponderant role in aquatic communities when introduced outside their original range (Vaeßen and Hollert 2015; Souty-Grosset et al. 2016); their detrimental effects have been linked to life history traits (early sexual maturity, high fecundity), wide tolerance to various environmental conditions, scarcity of natural predators, and competition with native crayfish (Gherardi 2006; Vaeßen and Hollert 2015). Due to the widespread diffusion of North American crayfish in Europe, many local cases of native species extinction have been observed (Kouba et al. 2014); the combination of biodiversity loss and ecosystem processes alteration often resulted in ecological and economic disruptions (Lodge et al. 2000).

The signal crayfish *Pacifastacus leniusculus* (Dana, 1852), native to the northwestern coast of the United States, is considered one of the most widespread and ecologically impactful decapod crustaceans introduced in Europe (Rebrina et al. 2015; Chucholl and Chucholl 2021). This species is a carrier of the crayfish plague, which is lethal for the most native European crayfish (Holdich et al. 2009). Moreover, its ecological niche largely overlaps with that of native crayfish (Préau et al. 2020); other detrimental effects are related to predatory activity and environmental alterations in the invaded areas. With its feeding habits, *P. leniusculus* modifies the food web and alters the composition of aquatic communities, consuming macrophytes, organic detritus, and preying on macroinvertebrates, amphibians and small benthic fish (Souty-Grosset et al. 2006; Procopio 2020). Furthermore, the digging activity associated with creating shelter, increases water turbidity, reducing light penetration with a consequent drop in primary productivity. Digging activity can also damage riverbanks, facilitating their collapse (Harvey et al. 2011). The species has led to the local extinction of many native crayfish populations in Scandinavia, England, and northern Europe (Lodge et al. 2000; Green et al. 2018). Even in Italy, as in other European countries, local extinction of the native crayfish *Austropotamobius pallipes* complex (Lereboullet, 1858) has been reported immediately after the introduction of the signal crayfish (Ghia et al. 2017); there are few cases in which a native population of *A. pallipes* complex was observed in syntopy with *P. leniusculus* (Ghia et al. 2019).

The distribution range of *P. leniusculus* in Italy, in the period 1981–2015, was limited to some watercourses in the northern part of the country (Capurro et al. 2007; Morpurgo et al. 2010; Bo et al. 2016; Ghia et al. 2017); the first report of the species in Central Italy dates back to 2020, when some individuals were found in the Clitunno River (Umbrian portion of the Tiber River basin) (Della Bella et al. 2021). The further spread of *P. leniusculus* in the Apennine watercourses raises serious concern because among all the North American crayfish, its ecological preferences largely overlap with that of *A. pallipes* complex (Souty-Grosset et al. 2006; Chucholl 2013), which being a cold-water species preferentially colonizes the upper river stretches (Bo et al. 2016).

According to the European Invasive Alien Species Regulation (EU 1143/2014), due to the detrimental multi-level impacts posed by the species on native biodiversity and ecosystem services, *P. leniusculus* is deemed as species of Union Concern. Therefore, population monitoring and quick removal actions shortly after its detection are required, to prevent a further spread of

the species. Based on these regulatory requirements, within the LIFE project (IMAGINE IPE/IT/000015) the Umbria Region prepared, an eradication plan that was started in 2022. The purpose of the eradication attempt was to contain the dispersal and the population growth of *P. leniusculus* in the Clitunno River Basin. The LIFE Programme is the EU's funding instrument for supporting Environment, Nature Conservation and Climate Action projects throughout the European Union (EU).

Two crucial traits expressing species fitness, population persistence and habitat adaptation are: population growth and demographic characteristics (Hoffmann et al. 2017). Thus, a deeper knowledge of these aspects plays a major role in crayfish invasions management (Guan and Wiles 1999), and it is essential for predicting potential range expansion of invasive species (Chuang and Peterson 2016).

Within this context, the present study aimed to i) test the effectiveness of the eradication actions, in terms of limiting *P. leniusculus* spread and population abundance in the Clitunno River Basin; and ii) assess distribution, age structure and growth of *P. leniusculus* population in the invaded area.

Material and methods

Study area

The Clitunno River (Tiber River Basin, central Italy) is a chalk stream that flows for 18 km, in an agricultural context, with an almost constant slope (0.18%), and stable thermal and hydrological regime (water temperature range: 11.8–20 °C; average flow rate: 1.5 m^3/s) (Lorenzoni et al. 2010, 2023). The stream originates from a rheo-limnocrenic spring system, called "Fonti del Clitunno". Despite the high cultural, naturalistic, and landscape value of the Clitunno River, which has inspired artists and poets since Roman times (Pascual et al. 2024), its riverbed is characterised by the presence of significant silt deposits that have built up over the years because of urban and industrial discharges (Lorenzoni et al. 2010). The Clitunno River has been classified as having "moderate ecological status", in line with the assessments carried out by the regional Environmental Protection Agency (ARPA Umbria) in the years 2016–2017 (Della Bella et al. 2021). The hydrographic network of the Clitunno River Basin is very complex and articulated, due to the numerous human-made hydraulic system modifications carried out over the centuries (Lorenzoni et al. 2023). The Clitunno River hosts an abundant macrophyte community (Cingolani et al. 2008), while amongst the most widespread fish species, several are currently facing the threat of extinction according to the IUCN Red List (IUCN 2024). These include *Anguilla anguilla* (Linnaeus, 1758) (CR), *Sarmarutilus rubilio* (Bonaparte, 1837) (VU), and *Barbus tyberinus* (EN). Additionally, according to the IUCN Red List of Italian Vertebrates (Rondinini et al. 2022), the cyclostome *Lampetra planeri* (Bloch, 1784) is listed as vulnerable. The native crayfish *A. pallipes* complex is locally extinct, while the invasive *Procambarus clarkii* (Girard, 1852) is widely distributed throughout the Basin, including the area considered in this study. Here the species seems to find suitable conditions for reproduction only in the downstream warmer river stretches (Lorenzoni, personal observation).

Experimental design

Fig. 1 schematically shows the selected area for *P. leniusculus* eradication activities, located in the upper part of the Clitunno River Basin. The eradication programme, conducted using both trapping and electrofishing, was based on the results of preliminary monitoring carried out in 2021 (Lorenzoni et al. 2023). The entire area has been divided into 13 stretches. Nine sites (from -1 to -9) were situated upstream from the first detection point of *P. leniusculus* (site 0); three sites, numbered from 1 to 3 and located downstream of site 0, were selected as "control sites", to check the possible range expansion, given that, in these areas, the species was not detected during the preliminary monitoring. The most abundant *P. leniusculus* population, occurring in the site identified as the "invasion core", was chosen to conduct the demographic and growth analyses. Table 1 shows the main environmental characteristics for each river stretch, including canopy cover (i.e. the percentage of vegetation coverage of the riverbed), prevalent substrate (i.e. the dominant particle size of the sediments), and shaded area (i.e. the percentage of riparian vegetation shading). In the present research we tested the hypothesis that the eradication actions were effective in limiting *P. leniusculus* spread and population abundance in the investigated area.

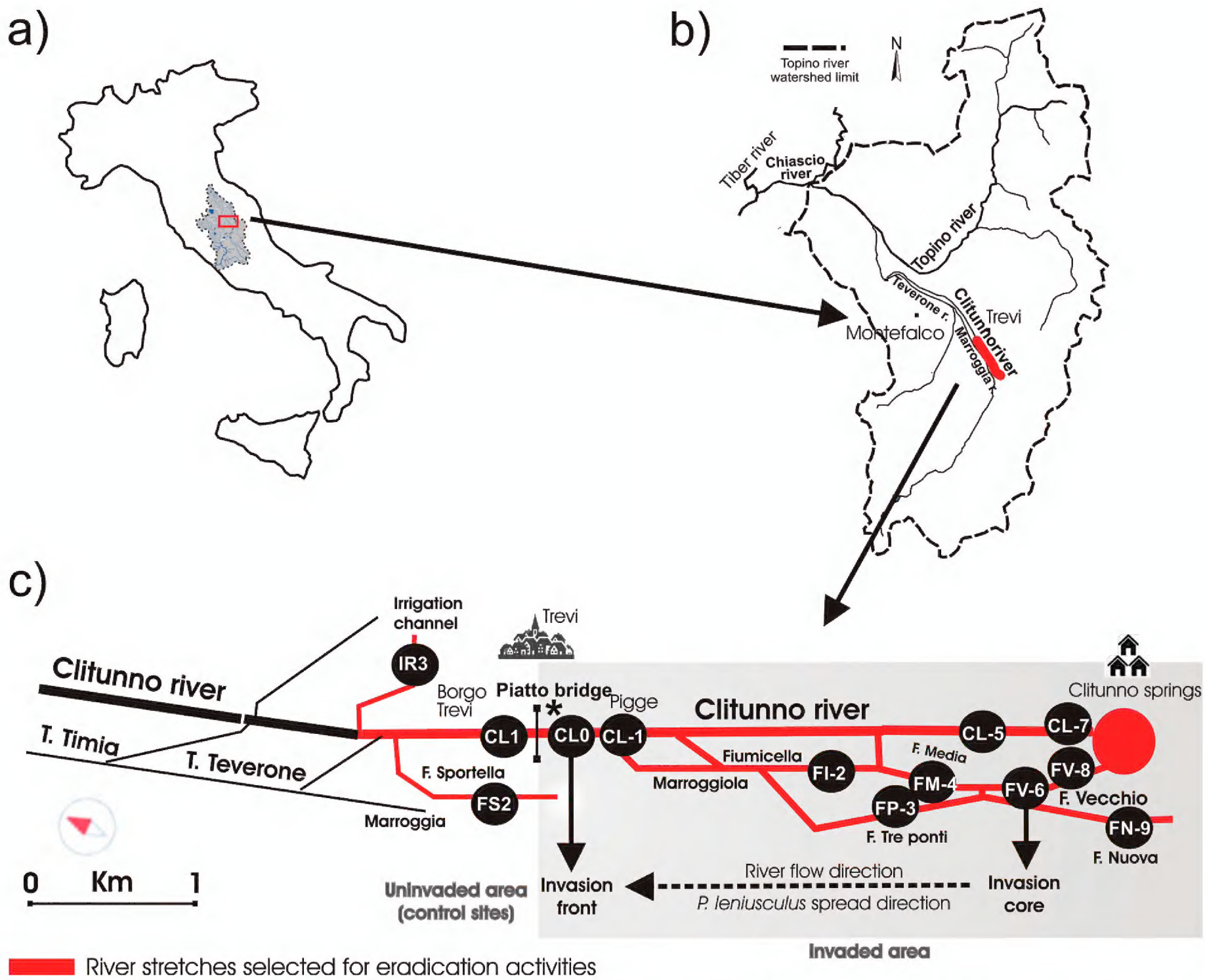

Figure 1. Location of the sites selected for *P. leniusculus* removal activities. **a.** Tiber River watershed; **b.** Topino River sub-watershed; **c.** Hydrographic network of the upper Clitunno River Basin and location of the eradicated stretches. An asterisk indicates the first detection site. The shaded area includes the species occurrence stretches. The dashed arrow indicates the spread direction of the signal crayfish.

Table 1. List of sampling sites along geographical coordinates, and main environmental features. Canopy cover, Prevalent substrate and Shaded area refer to: vegetation coverage of the riverbed, dominant particle size of the sediments, and riparian vegetation shading, respectively.

River stretches code and locality	Geographic coordinates	Average depth (m)	Average width (m)	Canopy cover (%)	Prevalent substrate	Shaded area (%)	Anthropisation level	Type of disturbance
CL0 Piatto Bridge **first detection site**	33 T 4747402.16 m N, 315656.63 m E	0.9	6	75	Silt	30	Very high	Canalisation, Diffuse organic pollution
CL1 Borgo Trevi	33 T 4749177.40 m N, 314823.23 m E	0.8	12	90	Silt	90	High	Canalisation, Diffuse organic pollution, Presence of obstacles
FS2 Pietrarossa	33 T 4750596.97 m N, 313932.25 m E	0.4	1	20	Silt	50	Very high	Canalisation, Point-source organic pollution
IR3 Borgo Trevi	33 T 4750793.29 m N, 314195.95 m E	0.5	2	10	Silt	10	Very high	Canalisation , Diffuse organic pollution
CL(-1) Pigge	33 T 4746511.93 m N, 316183.45 m E	0.7	12	70	Silt - Fine Gravel	10	Moderate	Canalisation
FI(-2) Pigge	33 T 4746497.39 m N, 316178.65 m E	0.7	2.5	30	Silt	20	Moderate	Canalisation, Diffuse organic pollution
FP(-3) Treponti	33 T 4745034.65 m N, 316789.89 m E	1	2.5	20	Silt	50	High	Canalisation, Diffuse organic pollution
FM(-4) Treponti	33 T 4745058.25 m N, 316802.64 m E	0.4	4	20	Silt - Fine Gravel	80	High	Canalisation, Diffuse organic pollution
CL(-5) Treponti	33 T 4745128.83 m N, 316837.82 m E	1	10	100	Silt - Sand	30	Moderate	Canalisation, Diffuse organic pollution
FV(-6) Treponti	33 T 4745008.15 m N, 316869.76 m E	0.5	3	80	Silt	80	High	Canalisation, Diffuse organic pollution
CL(-7) Fonti	33 T 4744708.80 m N, 317445.71 m E	1	8	90	Silt - Sand	70	Moderate	Canalisation
FV(-8) Fonti	33 T 4744664.53 m N, 317446.05 m E	0.3	2.5	20	Silt	100	Very high	Canalisation, Diffuse organic pollution, Presence of obstacles
FN(-9) Treponti	33 T 4744929.48 m N, 316845.18 m E	0.5	2.5	70	Silt	50	Very high	Canalisation, Point-source organic pollution

Removal activities

Baited traps and artificial refuge traps

The removal actions were conducted biweekly, from June 2022 to December 2024, using two types of traps: baited and artificial refuge traps (ART). Baited traps consisted of double entry cylindrical (50 × Ø 30 cm) plastic cages, with a mesh size of 0.5 cm. They were baited with dry food for cats or fish. In total, up to 22 baited traps were used, with an average value of 15. The traps were placed, approximately 25 m apart from each other, in a semi-submerged position along the banks, to avoid causing disturbance to non-target species that could be attracted by the bait, following indications reported in the signal crayfish National Management Plan (Tricarico et al. 2021).

Following Green et al. (2018), to collect more individuals representative of small size ranges, the use of baited traps was considered more effective in combination with ART. ARTs were made of seven PVC tubes, of which two of 10-, 20-, 32- diameter, respectively, and one of 50-mm diameter, and all 400 mm long, joined by a clamp, and closed by a fine mesh net at one end. Up to 10 ARTs were employed in total, with an average value of seven; they were placed close to baited traps, in the areas most frequented by juveniles.

The trend in catches over time was assessed by Catch per Unit Effort (CPUE), calculated as a daily average of catches per trap (baited and ART). As *P. leniusculus* is a species of Union concern, all individuals were frozen immediately after capture and all specimens were brought to the laboratory for sex determination and biometric measurements.

Electrofishing

Limited to wadable stretches, in all the 13 river stretches, the removal activities included electrofishing, using a 4.5 kW electro shocker. The interventions were carried out on a seasonal basis, excluding winter, applying a time of 45/60 minutes at each sampling site. The average length of the river stretches was 40 m (range: 15–120 m); they were sampled by at least three operators, proceeding from downstream to upstream. In some parts of the Clitunno River, where the water was too deep for electrofishing, the catches were made along the riverbanks.

Environmental characterisation

To investigate the environmental conditions that could most influence the presence and activity of the signal crayfish, at the same time as electrofishing, the following physico-chemical parameters were measured: water temperature (°C), pH (units), electric conductivity (μS cm^{-1}) and dissolved oxygen (mg l^{-1}). The measurements were conducted simultaneously with the electrofishing removal activities, using a multiparametric probe (Hanna Instruments, Padova, Italy).

Laboratory activities

For each individual identified as *P. leniusculus* based on morphological characters (i.e. white oval patch at the joint of the claw fingers and two pairs of post-orbital ridges), sex was determined, the total weight was measured using a digital scale (accuracy ± 0.1 g) and the following biometric parameters were measured using a calliper (accuracy ± 1 mm): cephalothorax length (CL) from the tip of the rostrum to the posterior edge of the carapace and the total length (TL) from the tip of the rostrum to the telson. Cheliped damage (absent or regenerating) or the presence of other macroscopic lesions were also recorded.

Demographic features and growth of *P. leniusculus* population

For the FV(-6) population, the total length-weight relationship (LWR) was estimated by the least-squares method (Ricker 1975), based on the logarithmic equation: $\log_{10} W (g) = a + b \log_{10} CL (cm)$, where a is the intercept on the Y-axis and b is the regression coefficient. The standard error was calculated for the slope (b) of LWR. Isometric growth was tested through a *t*-test, using the equation:

$$t_s = b\text{-}3/S_b$$

where S_b is the standard error of the slope (b), for $\alpha = 0.05$ (Sokal and Rohlf 1987). The LWR was fitted both to the total sample and separately for males and females.

Age classes were estimated by Bhattacharya's method based on the CL frequency distribution data (Bhattacharya 1967), using the software FiSAT II (FAO-ICLARM Stock Assessment Tools v. 1.2.2).

Theoretical growth was estimated by the von Bertalanffy growth curve model (VBGE) (von Bertalanffy 1938): $\mathbf{CLt = L_\infty(1 - e^{-k(t-t_0)})}$ where CL_t is the carapace length of the crayfish at time t, L_∞ the theoretical maximum carapace length (cm), k the rate of approach to L_∞ and t_0 the theoretical age (in years) at which

$CL_t = 0$. Furthermore, the index of growth performance (Φ') was calculated by the equation of Pauly and Munro (1984): $\Phi' = \log_{10} k + 2 \log_{10} L_\infty$ where k and L_∞ are the growth parameters of the von Bertalanffy model.

Statistical analysis

To test the differences between mean CPUE amongst sites, amongst years and the interaction between these variables (site x year), a two-way factorial ANOVA was performed. A one-way ANOVA was performed to compare physico-chemical parameters amongst sites. For LWR, differences between sexes were assessed by ANCOVA. To compare the length of *P. leniusculus* caught by different removal methods, a Kruskal-Wallis ANOVA was performed. The normality of length data distribution was tested by Shapiro–Wilk test. All statistical tests were conducted using Dell STATISTICA 13 software for Windows.

Results

Removal activities and *P. leniusculus* distribution

Baited traps and artificial refuge traps

A total of 180 removal activities were conducted. The overall effort applied through setting traps was equal to 2057 trap days in 2022, 5583 trap days in 2023, and 8708 trap days in 2024. Overall, 1075 *Pacifastacus leniusculus* individuals were removed for a total biomass of 37.9 kg. This species was only caught upstream from the first detection site (Fig. 1), where the largest sample (91.63% of total individuals) has been removed from Fosso Vecchio, a small tributary of the Clitunno upper stretch. In FV(-6) we observed a progressive decreasing trend over time of the mean annual CPUE values (± SE), which dropped from 0.284 ± 0.071 ind/trap day recorded in 2022 to 0.065 ± 0.005 in 2024 (Fig. 2). A drastic reduction of CPUE was also observed in the same period for FM(-4) and CL(-5), where the mean annual values (± SE) decreased from 0.070 ± 0.055 to 0.024 ± 0.008 and from 0.018 ± 0.061 to 0.001 ± 0.014 ind/trap day, respectively. At all other sites, no *P. leniusculus* individuals were captured in 2024 by traps. There are differences between the mean values amongst sites (two-way factorial ANOVA: $F = 32.19$; $P = 0.004$), and a significant interaction between these variables (two-way factorial ANOVA: $F = 2.51$; $P = 0.001$).

During the eradication interventions, a progressive significant decreasing trend in the average daily CPUE values was observed through time for traps in the FV(-6) (Fig. 3). The computed equation was $y = 2.83 - 0.025 \cdot x$ ($F = 6.56$; $P = 0.022$; $r^2 = 0.46$); the regression slope significantly differed from 0 ($t = 2.79$; df = 9; $P = 0.022$).

For baited traps, the analysis of the monthly trend in CPUE values allowed us to identify two peaks, occurring in April and August (Fig. 4), with mean values (± SE) recorded at 0.27 ± 0.05 and 0.21 ± 0.02, respectively. The lowest values were observed in January (mean ± SE = 0.02 ± 0.02) and February (mean ± SE = 0.02 ± 0.01). The differences amongst the mean values were statistically significant (one-way ANOVA: F = 2.69; $P = 0.003$). For ARTs, the monthly mean values were always lower than those of baited traps; the lowest values were observed in December (0.004 ± 0.003) and January (zero catches). However, in this case, the differences amongst the mean values were not statistically significant (one-way ANOVA: F = 1.74; $P = 0.08$).

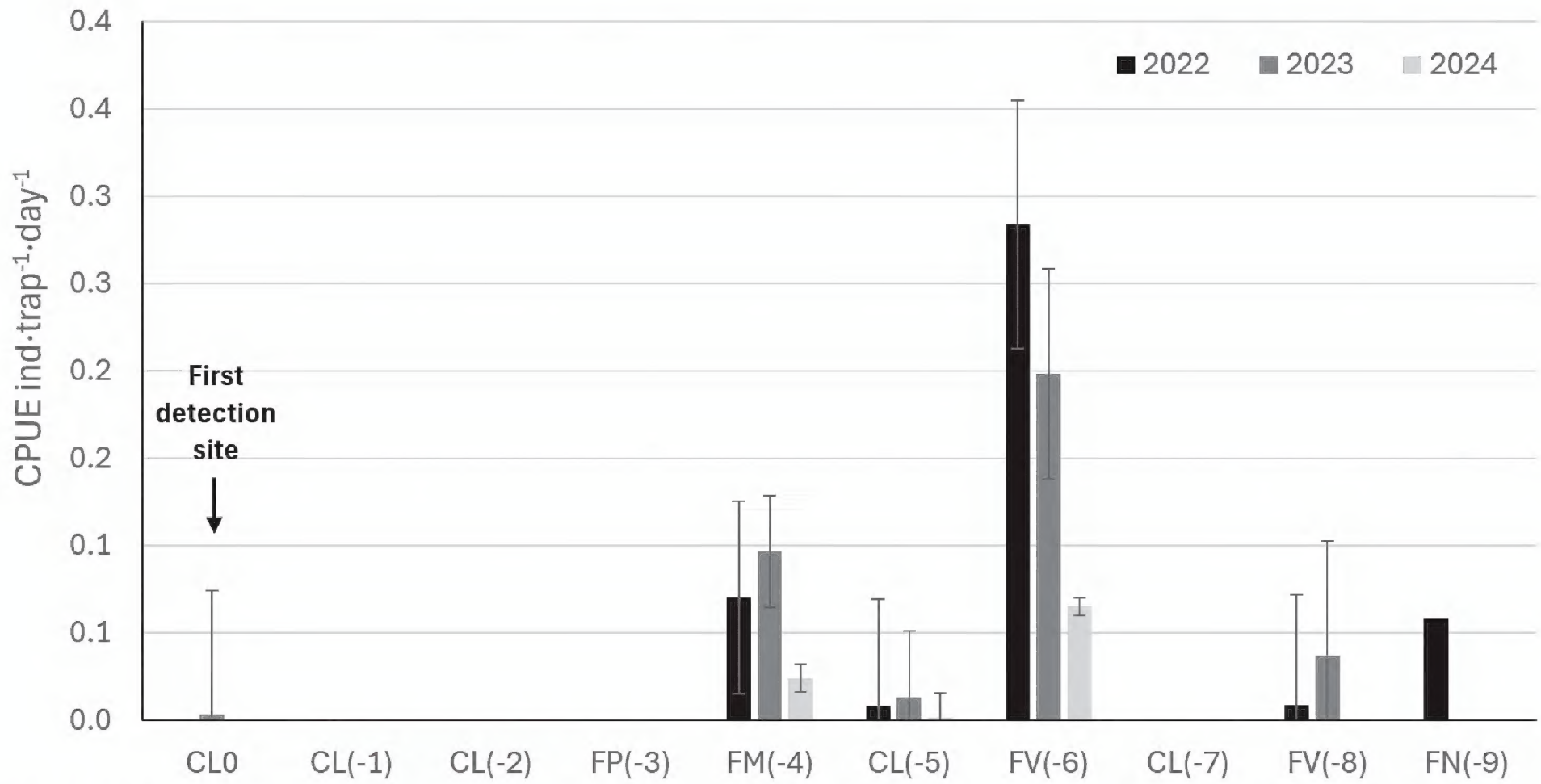

Figure 2. Baited traps and artificial refuge traps: mean annual CPUE values for *P. leniusculus* are broken down by year and removal sites falling within the invaded area. Vertical bars denote standard errors.

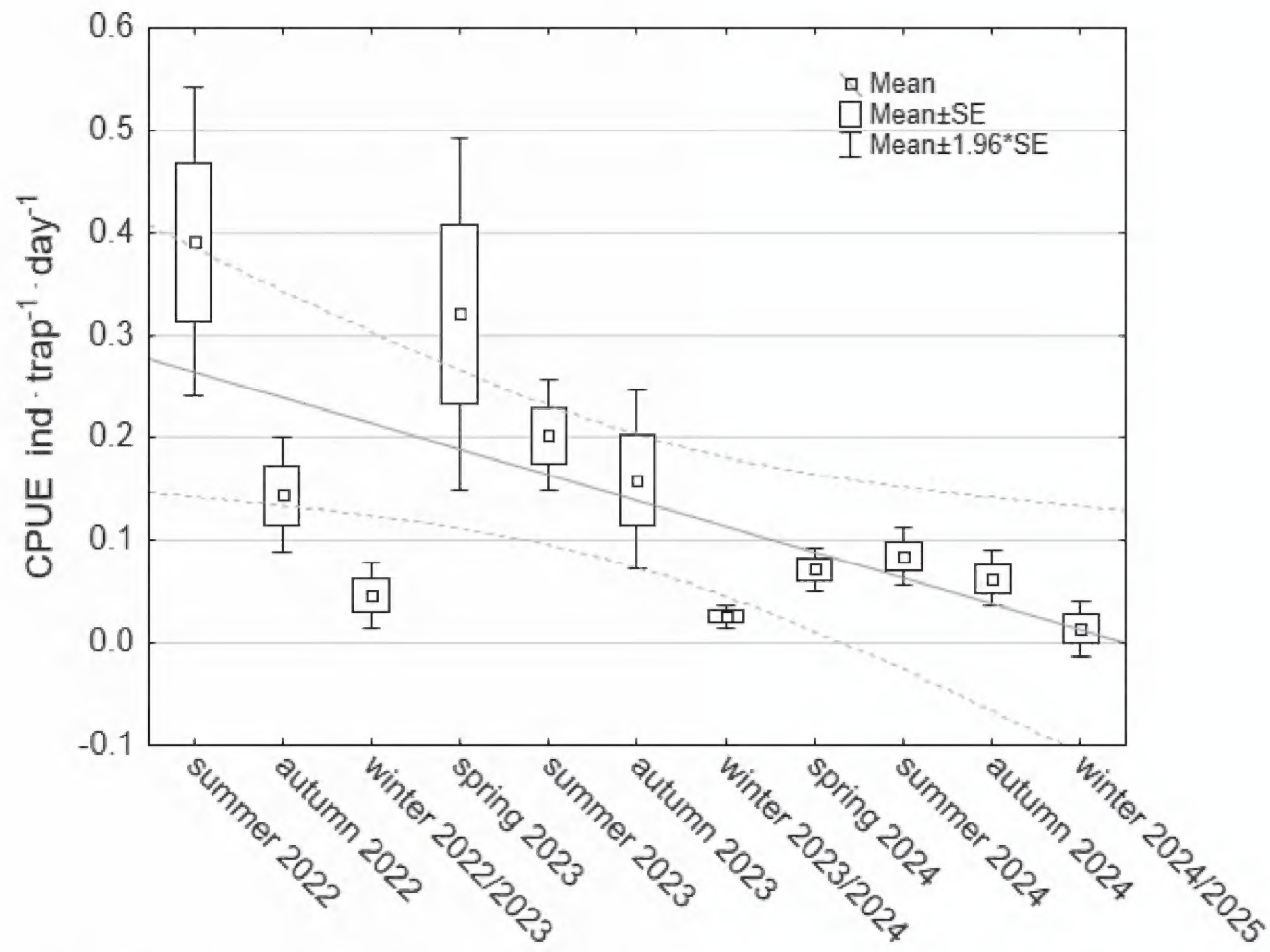

Figure 3. Trend over time in mean seasonally CPUE values for *P. leniusculus* during eradication interventions conducted with baited traps and artificial refuge traps in the Fosso Vecchio River. The solid line represents linear regression (equation: y = 2.83–0.0251*x; $P = 0.0218$; $r^2 = 0.46$). The dotted lines represent confidence limits (95%).

Electrofishing

A total of 79 electrofishing removals were carried out, during which overall 28 specimens were collected. The mean total length of individuals (± SE) was 10.0 ± 2.59 cm (range 4.3–13.5 cm); the mean weight (± SE) was 38.66 ± 23.77 g (range 2.3–72.0 g). This method proved its effectiveness in CL0 and FV(-6), with captures recorded exclusively in 2022 and 2023. In 2024, two individuals were also captured from FM(-4) (Fig. 5).

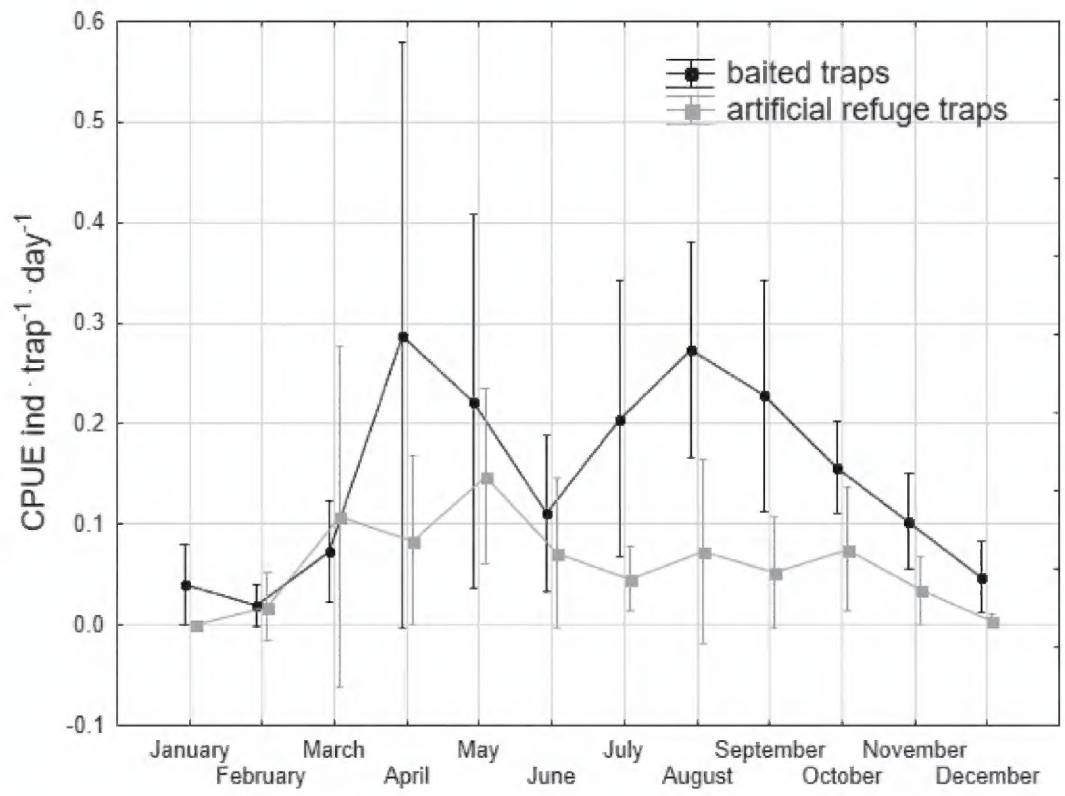

Figure 4. Monthly mean CPUE values for *P. leniusculus* during eradication interventions conducted with baited traps and artificial refuge traps in the Fosso Vecchio River. Vertical bars denote confidence limits.

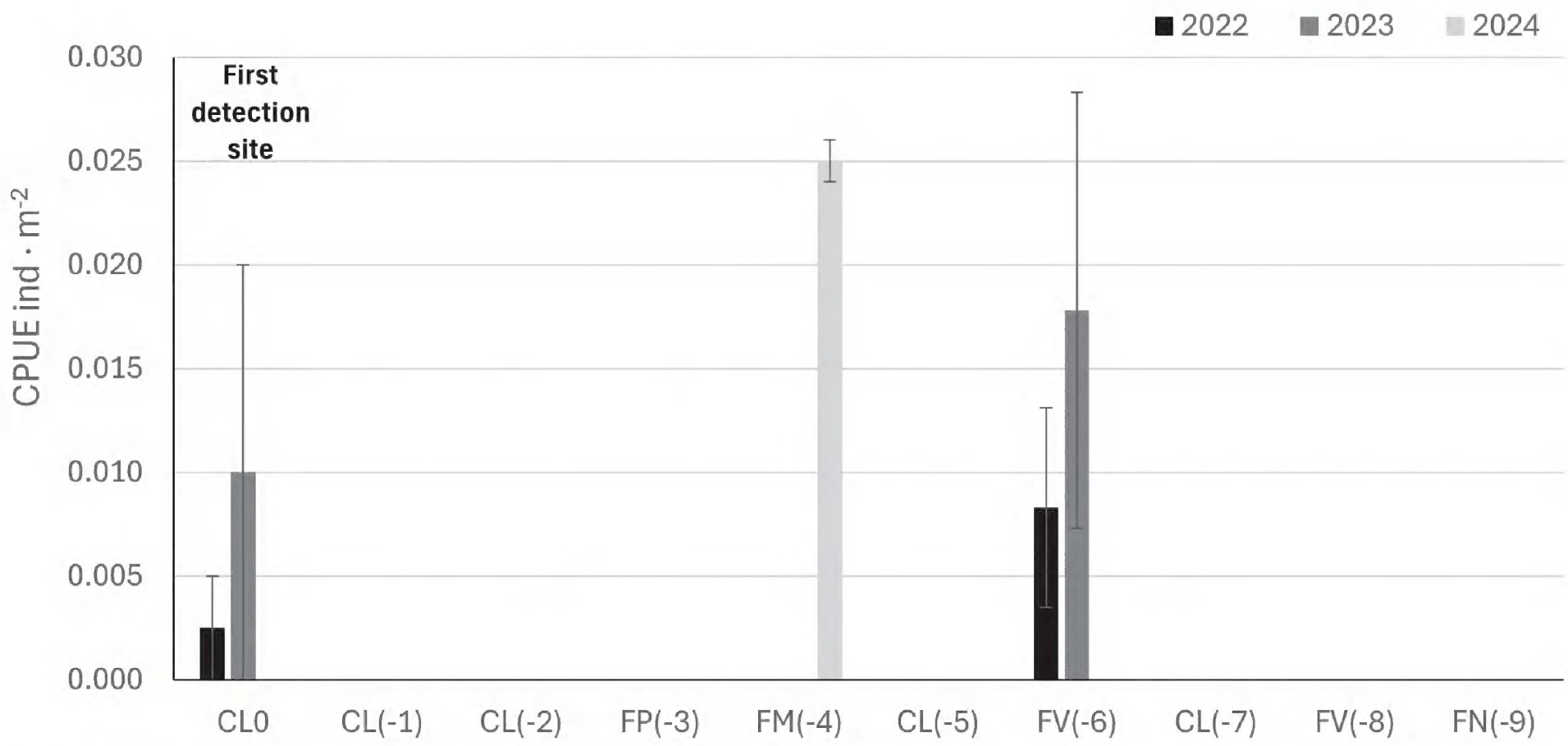

Figure 5. Electrofishing: mean annual CPUE values for *P. leniusculus* are broken down by year and removal sites falling within the invaded area. Vertical bars denote standard errors.

Selectivity of removal methods

The mean length (± SE) of individuals caught with ARTs (7.36 ± 0.27 cm) was significantly lower from those of both baited traps (10.04 ± 0.04 cm) and electrofishing (9.60 ± 0.58 cm) at the Kruskal-Wallis ANOVA ($\chi^2 = 38.44$; $P < 0.001$). Sex ratio did not differ significantly amongst different removal methods at the chi-square test ($\chi^2 = 1.10$; $P = 0.578$).

Environmental characterisation

Water temperature ranged from 11.20 °C, recorded in FM(-4), to 19.36 °C, recorded in FN(-9) (Fig. 6a). The differences between the mean values calculated for the 13 sites were statistically significant (one-way ANOVA: F = 5.27; $P < 0.001$).

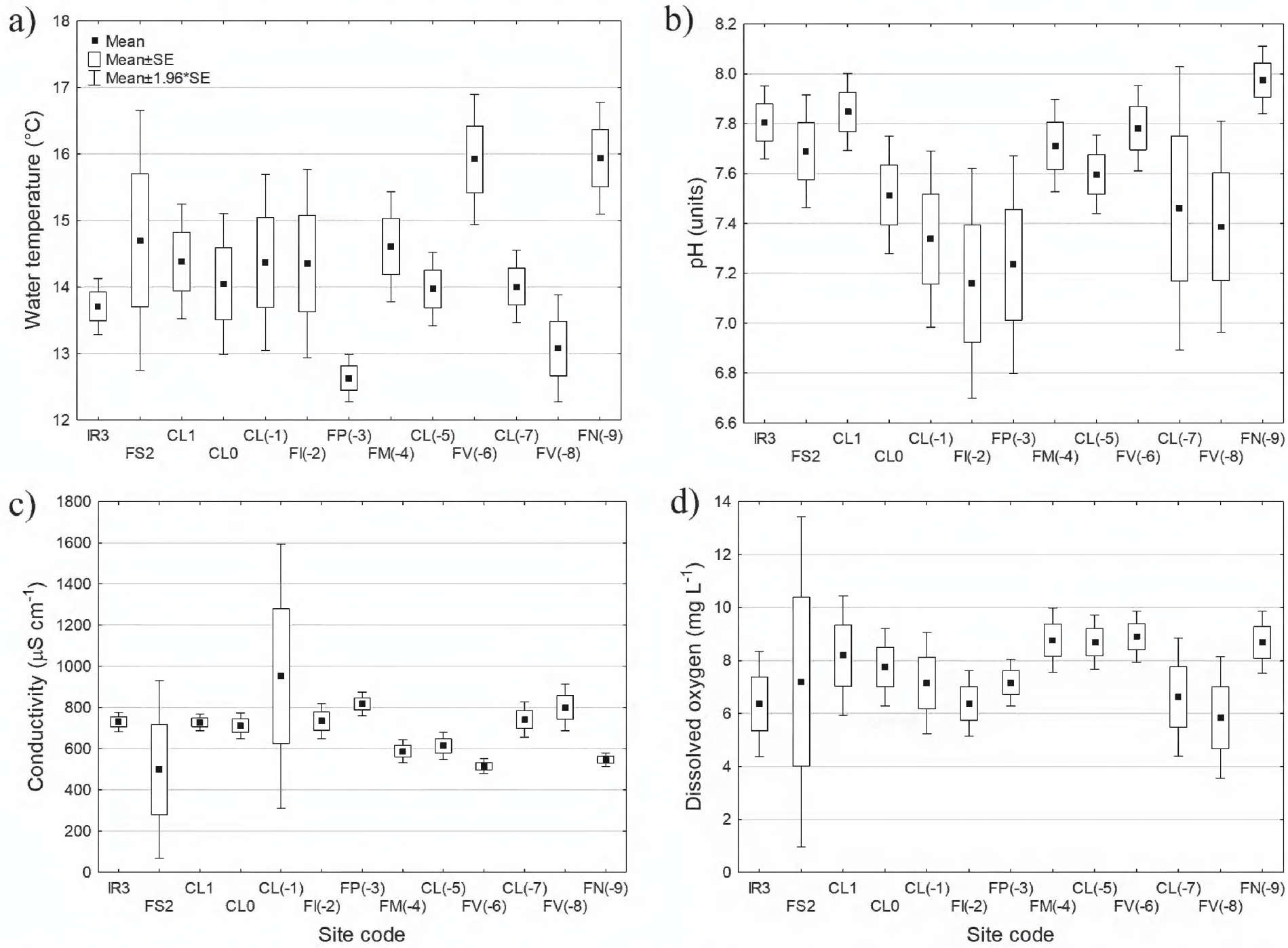

Figure 6. Box-plots of physico-chemical parameters collected during eradication activities carried out by electrofishing: **a.** Water temperature (n = 134); **b.** pH (n = 109); **c.** Conductivity (n = 113); **d.** Dissolved oxygen (n = 117).

The mean value (± SD) recorded for FV(-6) (13.97 ± 1.29 °C) was statistically different from the mean values recorded for CL(-5) (12.63 ± 0.77 °C; $P = 0.015$), FN(-9) (15.92 ± 2.28 °C; $P = 0.001$) and FP(-3) (15.94 ± 1.61 °C $P = 0.001$) as revealed by the Fisher LSD post hoc test.

FI(-2) presented the lowest pH value (6.54 units), while the highest value was measured in FN(-9) (8.39 units) (Fig. 6b). The differences between the mean values were statistically significant (one-way ANOVA: $F = 2.69$; $P = 0.004$). The average value (± SD) observed in FV(-6) (7.60 ± 0.32) was significantly different from those observed in CL(-5) (7.23 ± 0.83; $P = 0.024$) and FP(-3) (7.98 ± 0.22; $P = 0.033$), as determined by the Fisher LSD post hoc test.

Conductivity values covered a wide range, from 79 µS cm^{-1}, measured in FS2, to 2254 µS cm^{-1}, measured in CL(-1) (Fig. 6c). The mean value differences amongst sites were statistically significant (one-way ANOVA: $F = 4.08$; $P < 0.001$). The mean value (± SD) observed for FV(-6) (614.35 ± 141.21 µS cm^{-1}) exhibited a statistically significant difference when compared to the mean values calculated for CL(-1) (952.40 ± 0731.65 µS cm^{-1}; $P = 0.001$) and CL(-5) (817.40 ± 114.33 µS cm^{-1}; $P = 0.002$), as determined by the Fisher LSD post hoc test.

Dissolved oxygen varied from 1.42 mg/l, recorded in FS2, to 14.80 mg/l, recorded in FM(-4) (Fig. 6d). The differences between the mean values calculated for sites were not statistically significant (one-way ANOVA: $F = 1.56$; $P = 0.115$).

Demographic features and growth

The total length of crayfish ranged from 1.1 to 14.8 cm (mean = 9.82 ± 0.06 SE) and weight from 0.025 to 118.70 g (mean = 36.0 5 ± 0.60 SE). The largest size recorded was 14.8 cm in total length for females and 13.9 cm for males. Females represented 50.4% of the total sample; sex ratio (1: 1.1 males/females) did not differ significantly from the theoretical 1:1 value ($\chi^2 = 0.004$; $P > 0.05$). For both sexes of FV(-6) population, a total of six cohorts, extended with continuity from 0+ to 5+, were identified, based on the carapace length frequency distribution data (Fig. 7).

The LWR equations for males and females were: $\log_{10} W = -0.68 + 3.26 \log_{10} CL$ ($R^2 = 0.98$; $P < 0.001$), and $\log_{10} W = -0.61 + 3.11 \log_{10} CL$ ($R^2 = 0.98$; $P < 0.001$), respectively (Fig. 8). In both equations the b slopes were significantly higher than three (df = 387; $t = 11.95$; $P < 0.001$ for males; df = 395; $t = 4.82$; $P < 0.001$ for females), indicating positive allometric growth (Ricker 1975). The LWRs calculated for both sexes were statistically different (ANCOVA: $F = 71.74$; $P < 0.001$). Based on this latter result, the growth of males and females was analysed separately.

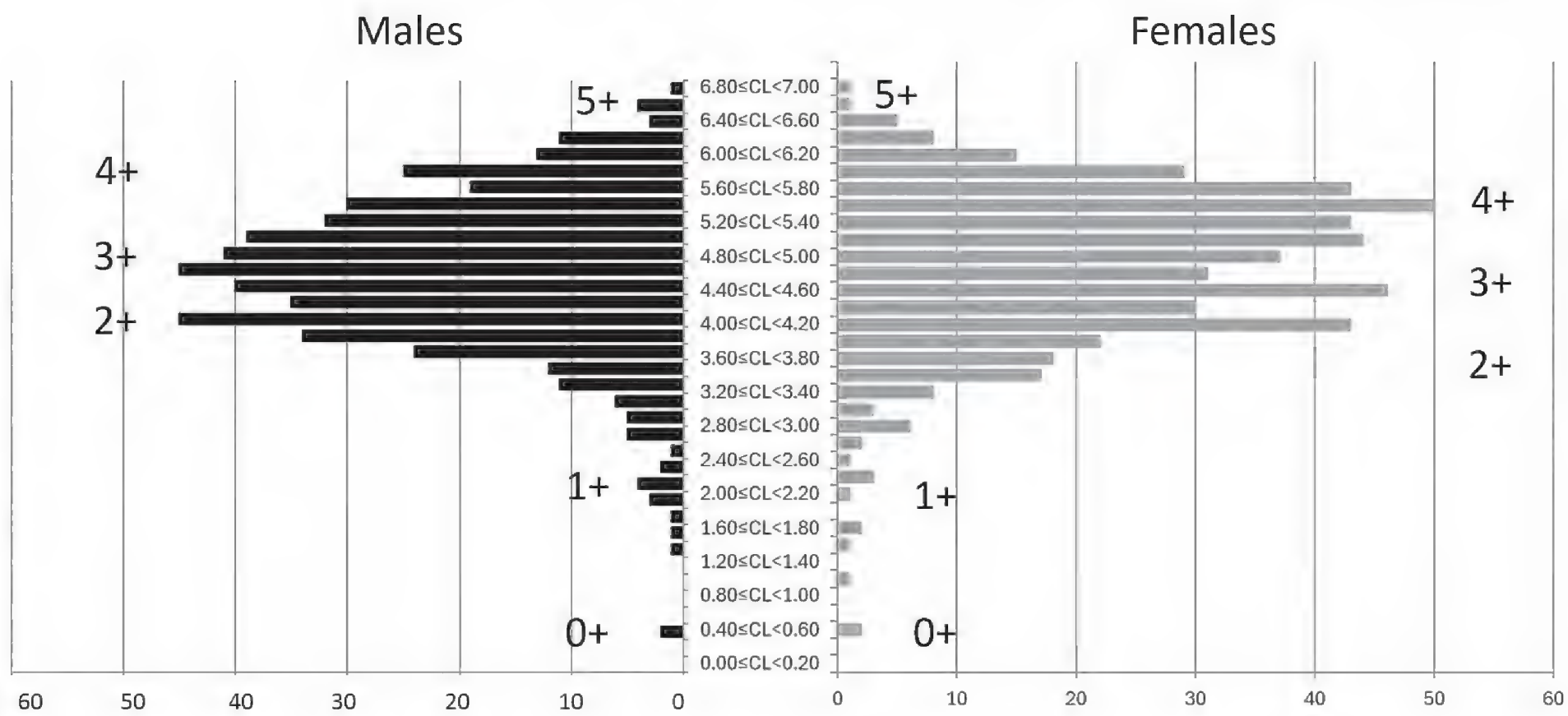

Figure 7. Frequency chart of carapace length for the total sample of males (black bars) and females (grey bars).

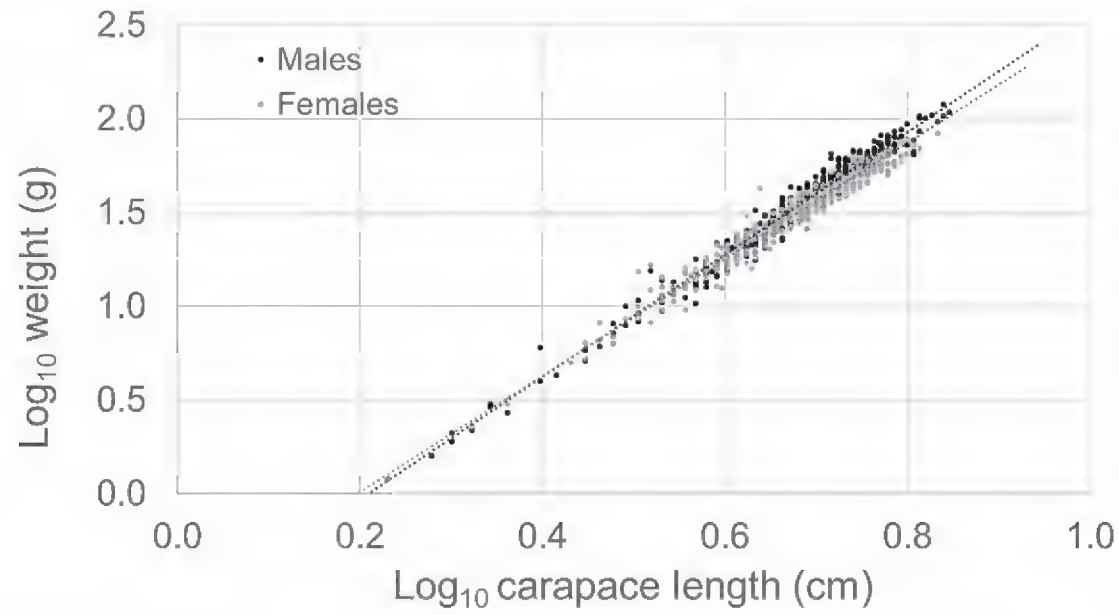

Figure 8. LWRs for *P. leniusculus* population of Fosso Vecchio River. Black and grey dots represent males and females, respectively. The dotted lines represent linear regressions for males (equation: y = 3.2629x - 0.6826; $r^2 = 0.9824$), and females (equation: y = 3.105x - 0.6103; $r^2 = 0.981$). ANCOVA: F = 70.74; $P = 0.001$).

Figure 9. VBGEs for *P. leniusculus* calculated for females (grey) (equation: CLt = 7.51(1 – e–0.40 (t+0.0)); Φ'=1.35) and males (black) (equation: CLt = 8.50(1 – e–0.28 (t+0.25)); Φ '=1.30).

The VBGEs calculated for females and males were $CLt = 7.51\ (1 - e^{-0.40(t)})$ and $CLt = 8.50\ (1 - e^{-0.28(t + 0.23)})$, respectively. The parameters obtained for the theoretical growth highlighted a better performance of females (Φ' = 1.35), showing a lower theoretical carapace length ($L\infty$ = 7.51 cm) than males ($L\infty$ = 8.50 cm), but a faster growth rate (k = 0.40) (Fig. 9).

Discussion

Our research provided evidence that, within a short period from its first detection, *P. leniusculus* has become naturalized in the Clitunno River Basin, rapidly resulting in a self-sustaining population mainly confined in a small tributary (FV(-6)). Through intensive removal efforts, a drastic reduction in the population abundance was achieved over nearly three years of eradication activities. Additionally, concurrent monitoring conducted at control sites demonstrated that the species has not further extended its invasive range in this system.

The distribution analysis revealed that the current invasive range of the species covered 3.5 km of the Clitunno hydrographic network, extending from the springs downstream. The established and most abundant population in FV(-6) could be identified as the "invasion core" (i.e. where the species was probably initially introduced and established a stable population, *sensu* Carvalho et al. (2025)), while the CL0 distribution point could be considered the downstream "invasion front" (i.e. invasion edge), with much lower abundance than the core area. This is not a surprising result, considering the high dispersal rate of the species, which can reach 6.4–24 km/yr (Hudina et al. 2009; Bernardo et al. 2011; Anastácio et al. 2015). Various authors have reported both downstream and upstream *P. leniusculus* invasion range expansions, through active and passive movements, including secondary introductions (Anastácio et al. 2015; Rebrina et al. 2015; Carvalho et al. 2025). Active movements could be driven both by environmental conditions (thermal conditions, food availability) and biological factors (high population density, occurrence of other crayfish species) (Rebrina et al. 2015). Passive movements have

been related to high flows (Rebrina et al. 2015), and to the potential transport on dry land by waterbirds (Águas et al. 2014) and off-road vehicles (Banha et al. 2014). The high desiccation survival capacities of *P. leniusculus* favour both active and passive overland dispersal, including human translocations (Banha and Anastácio 2014). Amongst all environmental drivers, water temperature is an important factor influencing active dispersal and colonisation pattern of the species (Marques et al. 2015). Also, in the case of the Clitunno River Basin, where the environmental characteristics of watercourses are quite stable over time, but more heterogeneous in space, water temperature probably played an important role in the establishment process and dispersal patterns of *P. leniusculus*. The physico-chemical characterisation revealed the presence of significant differences between the mean temperatures of the FV(-6) and the adjacent sites, even if the thermal conditions were always still within the tolerance range (4–25 °C) reported for the species (Shimizu and Goldman 1983). Another key element in the invasion process could be represented by the co-occurrence with *P. clarkii*, which can potentially exert competitive pressure, even if, in some studies, the two species have shown diversifications in terms of thermal preferences and habitat use (Henttonen and Huner 1999; Bernardo et al. 2011; Anastácio et al. 2015). Even the dissolved oxygen concentration and, in general, the human impact level and water pollution that characterises the different sites, could justify the different distribution of *P. leniusculus* within the investigated area. Further analyses are required to evaluate these hypotheses.

Despite a fairly widespread distribution and a well-structured population, this study found that the relative abundance of the Clitunno River Basin population of *P. leniusculus* was not particularly high, except for a few localised areas. This is in contrast with what is reported in literature for other Italian invaded areas where maximum CPUE values are of > 4 ind/trap day (Capurro et al. 2009; Ghia et al. 2017). This condition probably played a crucial role in the success of the eradication efforts.

The potential competition with *P. clarkii* may significantly influence the presently low population density of *P. leniusculus* in the Clitunno River and could also be crucial in predicting future distribution patterns of both species. In-depth studies on this aspect are currently ongoing.

The greatest removal efforts have been concentrated on the invasion core FV(-6), where the average CPUE values and the number of crayfish removed by traps were much higher than elsewhere. Here, the average CPUE values of traps showed a significant decreasing trend over time, arguing in favour of the removal actions' effectiveness. The trend of catches over time highlighted the presence of peaks concentrated in the spring and summer periods, when *P. leniusculus* is most active, due to the strong positive relationship between water temperature and crayfish movements (Bubb et al. 2002). This result could also reflect moulting activities (Shimizu and Goldman 1983). In winter, catches were low, but crayfish never completely disappeared. The peak in catches occurring in late summer was also probably due to the intensification of crayfish movements close to the reproductive period.

The outcomes of electrofishing confirmed the field-effectiveness of this method in the capture of crayfish and the limited number of specimens obtained may be linked to the high abundance of shelters that provided opportunities for crayfish to escape capture. One additional limitation pertains to the selectivity of the method, which, like baited traps, tends to favour the capture of larger individuals. In contrast, the application of ARTs has demonstrated effectiveness in capturing juvenile crayfish (i.e. TL < 6 cm), confirming the findings of Green et al. (2018).

The overall sample of *P. leniusculus* caught during the removal actions was quite large, well distributed in size classes and balanced in the sex ratio. Maximum size (TL = 14.8 cm) was consistent with what has been reported for another Italian population (Capurro et al. 2007), but greater than some native Californian ones, for which a maximum CL of 4.28 cm was found (Shimizu and Goldman 1983). The analysis of the population structure highlighted the prevalence, in the overall sample, of sexually mature individuals, with TL > 6 cm (Souty-Grosset et al. 2006). The overall effectiveness of removal actions for young individuals seems to be limited, even with the implementation of ARTs. This factor should be considered in the ongoing efforts related to eradication initiatives. The presence of six cohorts (from 0+ to 5+), including the young-of-the-year (0+), suggested that the species had already been present in the Clitunno River Basin for several years. The number of age classes and the maximum size, the positive allometric growth (with significant difference between sexes), and reaching a mean carapace length equal to 7 cm at the age of six years are consistent with the findings of Guan and Wiles (1999) for British invaded lowland rivers. The rapid growth rate, observed especially for females, and the theoretical maximum length achievable, which approaches the maximum size reported for the species (i.e. 20 cm, according to Tucker and Tucker 2019), could be related to the availability of resources. The watercourses considered in this study are rich in food sources for crayfish, especially in relation to periphyton and macroinvertebrates. These have been reported to represent the main components of *P. leniusculus* diet in another Italian stream (Ercoli et al 2021). Population growth rate is often rapid when species initially colonise new environments with adequate resources; however, this growth tends to decelerate as population density rises and resources become constrained (Guan and Wiles 1996). For instance, significantly higher growth rates were observed for *P. leniusculus* during the initial four years following its release into a lake in southern Britain (Richards 1983; Hogger 1986). Furthermore, it should be considered that eradication activities have progressively reduced the size of the population, making intraspecific competition less likely, thus reducing pressure on juveniles, and influencing growth as a density-dependent mechanism (Holdich et al. 1999). Eliminating larger individuals may enhance the success of eradication efforts and reduce the spread of the species (Moorhouse and Macdonald. 2011); however, it could also result in increased survival rates, an earlier onset of sexual maturity, and a higher production of eggs (Holdich et al. 1999; Gherardi et al. 2011). For these reasons, the combination of different traditional and emerging eradication techniques (e.g. physical removal and release of sterilised individuals) (Manfrin et al. 2019) or the use of crayfish predators for biological control (Matos et al. 2025) could help to achieve more effective and long-lasting results.

Based on population structure and the already quite widespread diffusion, it is suggested that the species has been present in the Clitunno for some time, and that its introduction preceded its first detection. This is consistent with the existence of a "lag phase" during which the species was present but given the low population abundance it escaped detection. The most plausible explanation for *P. leniusculus* introduction was the escape of individuals from submerged cages, intended for the collection of live crayfish for catering purposes. The potential for continued propagule pressure through this pathway is quite low, thanks to the intervention of the competent authorities who carry out the necessary checks. Any further propagation of the species downstream will instead likely be linked to passive movements,

assuming that the environmental conditions favourable to the establishment of the species exist. Reconstructing the dispersal patterns of invasive species and the dynamics of the invasion process is crucial for the effectiveness of management strategies aimed at mitigating their further spread (Hudina et al. 2017; Carvalho et al. 2025). This analysis facilitates: i) the containment of ongoing invasions by alleviating the propagule pressure (Simberloff 2009), ii) the reduction of the likelihood that existing populations could serve as sources for secondary introductions (Bertelsmeier and Keller 2018), and iii) the minimisation of recolonisation chances following successful eradication efforts (Britton et al. 2011). It is crucial to implement prolonged monitoring across all regions affected by the species, particularly in areas where native crayfish exist (Kirjavainen and Westman 1999). This is necessary due to the current lack of comprehensive information regarding the population dynamics of *P. leniusculus* within its invaded range (Kirjavainen and Westman 1999).

Conclusions

Even though it is unlikely that invasive crayfish can be completely eradicated within a system where acclimatization has taken place, our findings show how effective control measures can be at limiting population abundance and preventing the alien species from spreading further. Early detection and removal actions conducted at small scale, applying high catch effort, could represent effective management tools to counteract the spread of invasive crayfish. Our results allowed us to improve the removal effectiveness by concentrating the capture efforts more on the most favourable periods (spring and late summer) and areas (FV-6). Additional research is required to clarify the environmental and biological factors (e.g. water chemistry and the co-occurrence with *P. clarkii*) that might affect the distribution of the species within the study area. This will help to determine if the conditions are conducive to further expansion or if the species has already reached its maximum capacity to invade all potentially suitable locations.

The control programme for *P leniusculus* in the Clitunno River ended in December 2024. However, it is intended to continue in the coming years, according to an adaptive management approach in which the objectives will be recalibrated based on the results of this study. In particular, it is planned that traps and electrofishing are integrated with other alternative techniques, such as male sterilisation or removing female pleopods (Ghia et al. 2024).

Acknowledgements

The authors are also grateful to the anonymous reviewers and the editor Pedro Anastácio for their constructive comments which have considerably improved the original version of the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

Funding

This study was supported and funded by LIFE IMAGINE – LIFE19 IPE/IT/000015 within the framework of the European Commission Life Projects.

Author contributions

Conceptualization: ML, AC. Data curation: FZ. Formal analysis: AC. Funding acquisition: ML. Investigation: FZ, FL, ML. Methodology: ML. Supervision: ML. Writing - original draft: AC. Writing - review and editing: FL, ML.

Author ORCIDs

Antonella Carosi https://orcid.org/0000-0003-0513-7287

Massimo Lorenzoni https://orcid.org/0000-0002-8548-9487

Data availability

All of the data that support the findings of this study are available in the main text.

References

Águas M, Banha F, Marques M, Anastácio PM (2014) Can recently-hatched crayfish cling to moving ducks and be transported during flight? Limnologica 48: 65–70. https://doi.org/10.1016/j.limno.2014.07.001

Anastácio PM, Banha F, Capinha C, Bernardo JM, Costa AM, Teixeira A, Bruxelas S (2015) Indicators of movement and space use for two co-occurring invasive crayfish species. Ecological Indicators 53: 171–181. https://doi.org/10.1016/j.ecolind.2015.01.019

Banha F, Anastácio PM (2014) Desiccation survival capacities of two invasive crayfish species. Knowledge and Management of Aquatic Ecosystems 413: 01. https://doi.org/10.1051/kmae/2013084

Banha F, Marques M, Anastácio PM (2014) Dispersal of two freshwater invasive macroinvertebrates, *Procambarus clarkii* and *Physella acuta*, by off-road vehicles. Aquatic Conservation 24(5): 582–591. https://doi.org/10.1002/aqc.2453

Bernardo JM, Costa AM, Bruxelas S, Teixeira A (2011) Dispersal and coexistence of two non-native crayfish species (*Pacifastacus leniusculus* and *Procambarus clarkii*) in NE Portugal over a 10-year period. Knowledge and Management of Aquatic Ecosystems 28(401): 28. https://doi.org/10.1051/kmae/2011047

Bertelsmeier C, Keller L (2018) Bridgehead effects and role of adaptive evolution in invasive populations. Trends in Ecology & Evolution 33(7): 527–534. https://doi.org/10.1016/j.tree.2018.04.014

Bhattacharya CG (1967) A simple method of resolution of a distribution into Gaussian components. Biometrics 23(1): 115–135. https://doi.org/10.2307/2528285

Bo T, Candiotto A, Delmastro GB, Fea G, Fenoglio S, Ghia D, Gruppuso L (2016) Prima segnalazione del gambero alloctono *Pacifastacus leniusculus* (Decapoda, Astacidae) in Provincia di Savona, Italia. Natural History Sciences. Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano 3(1): 63–65. https://doi.org/10.4081/nhs.2016.284

Britton JR, Gozlan RE, Copp GH (2011) Managing non-native fish in the environment. Fish and Fisheries 12(3): 256–274. https://doi.org/10.1111/j.1467-2979.2010.00390.x

Bubb DH, Lucas MC, Thom TJ (2002) Winter movements and activity of signal crayfish *Pacifastacus leniusculus* in an upland river, determined by radio telemetry. Hydrobiologia 483(1–3): 111–119. https://doi.org/10.1023/A:1021363109155

Capurro M, Galli L, Mori M, Salvidio S, Arillo A (2007) The signal crayfish, *Pacifastacus leniusculus* (Dana, 1852) [Crustacea: Decapoda:Astacidae], in the Brugneto Lake (Liguria, NW Italy). The beginning of the invasion of the River Po watershed. Aquatic Invasions 2(1): 17–24. https://doi.org/10.3391/ai.2007.2.1.2

Capurro M, Galli L, Mori M, Arillo A. (2009). Struttura della popolazione del gambero alloctono *Pacifastacus leniusculus* (Crustacea, Decapoda, Astacidae) nel Lago del Brugneto (Liguria, Italia nord occidentale). Biologia Ambientale 23(1): 13–20.

Carvalho F, Alves H, Pascoal C, Castro P, Miranda F, Teixeira A, Cássio F, Sousa R (2025) Invasive dynamics of the signal crayfish *Pacifastacus leniusculus* in a protected area. Hydrobiologia 852(3): 705–720. https://doi.org/10.1007/s10750-024-05717-w

Chuang A, Peterson CR (2016) Expanding population edges: Theories, traits, and trade-offs. Global Change Biology 22(2): 494–512. https://doi.org/10.1111/gcb.13107

Chucholl C (2013) Feeding ecology and ecological impact of an alien 'warm-water' omnivore in cold lakes. Limnologica 43(4): 219–229. https://doi.org/10.1016/j.limno.2012.10.001

Chucholl F, Chucholl C (2021) Differences in the functional responses of four invasive and one native crayfish species suggest invader-specific ecological impacts. Freshwater Biology 66(11): 2051–2063. https://doi.org/10.1111/fwb.13813

Cingolani L, Lazzerini G, Padula R (2008) Metodologia per l'individuazione e valutazione dei possibili impatti su un ecosistema fiume, derivanti da interventi di ripristino ambientale e di restauro della continuità fluviale. Arpa Umbria 1–90. https://www.arpa.umbria.it/au/clitunno/1%20FASE.pdf

Della Bella V, Mauro N, Tricarico E (2021) Prima segnalazione in Umbria del gambero della California *Pacifastacus leniusculus* (Dana, 1852), specie esotica invasiva di interesse unionale. Biologia Ambientale 35: 12–17. https://doi.org/10.30463/es211.002

Ercoli F, Ghia D, Gruppuso L, Fea G, Bo T, Ruokonen TJ (2021) Diet and trophic niche of the invasive signal crayfish in the first invaded Italian stream ecosystem. Scientific Reports 11(1): 8704. https://doi.org/10.1038/s41598-021-88073-2

Ficetola GF, Siesa ME, Padoa-Schioppa E, De Bernardi F (2012) Wetland features, amphibian communities and distribution of the alien crayfish, *Procambarus clarkii.* Alytes 29(1–4): 75–87.

Gherardi F (2006) Crayfish invading Europe: The case study of *Procambarus clarkii.* Marine and Freshwater Behaviour and Physiology 39(3): 175–191. https://doi.org/10.1080/10236240600869702

Gherardi F (2007) Understanding the impact of invasive crayfish. In: Gherardi F (Ed.) Biological invaders in inland waters: Profiles, distribution, and threats. Springer Netherlands (Dordrecht), 507–542. https://doi.org/10.1007/978-1-4020-6029-8_28

Gherardi F (2012) Control and management of non-indigenous crayfish. In: Reynolds J, Souty-Grosset C (Eds) Management of freshwater biodiversity: crayfish as bioindicators. Cambridge University Press (Cambridge), 195–197. https://doi.org/10.1017/CBO9781139031790

Gherardi F, Aquiloni L, Dieguez-Uribeondo J, Tricarico E (2011) Managing invasive crayfish: Is there a hope? Aquatic Sciences 73(2): 185–200. https://doi.org/10.1007/s00027-011-0181-z

Ghia D, Fea G, Gruppuso L, Bo T, Candiotto A, Fenoglio S, Sacchi R (2017) Distribution and establishment of the invasive signal crayfish *Pacifastacus leniusculus* in the Valla Stream (N-W Italy). Italian Journal of Freshwater Ichthyology 4: 101–108.

Ghia D, Fea G, Ventimiglia M, Capurro M, Oneto F, Ottonello D, Bo T, Candiotto A, Sacchi R (2019) Il gambero autoctono italiano e il gambero della California coesistono in un tratto del torrente Valla (Italia nord-occidentale). Italian Journal of Freshwater Ichthyology 5(1): 120–131.

Ghia D, Morabito S, Fea G, Ercoli F, Sacchi R (2024) A new alternative technique for sterilising invasive crayfish: Removing female pleopods did not alter courtship pheromone release in signal crayfish. Knowledge and Management of Aquatic Ecosystems 425(425): 16. https://doi.org/10.1051/kmae/2024016

Green N, Bentley M, Stebbing P, Andreou D, Britton R (2018) Trapping for invasive crayfish: Comparisons of efficacy and selectivity of baited traps versus novel artificial refuge traps. Knowledge and Management of Aquatic Ecosystems 419(419): 15. https://doi.org/10.1051/kmae/2018007

Guan R, Wiles PR (1996) Growth, density and biomass of crayfish, *Pacifastacus leniusculus*, in a British lowland river. Aquatic Living Resources 9(3): 265–272. https://doi.org/10.1051/alr:1996030

Guan RZ, Wiles PR (1999) Growth and reproduction of the introduced crayfish *Pacifastacus leniusculus* in a British lowland river. Fisheries Research 42(3): 245–259. https://doi.org/10.1016/S0165-7836(99)00044-2

Harvey GL, Moorhouse TP, Clifford NJ, Henshaw AJ, Johnson MF, Macdonald DW, Reid I, Rice SP (2011) Evaluating the role of invasive aquatic species as drivers of fine sediment-related river management problems: The case of the signal crayfish (*Pacifastacus leniusculus*). Progress in Physical Geography 35(4): 517–533. https://doi.org/10.1177/0309133311409092

Henttonen P, Huner JV (1999) The introduction of alien species of crayfish in Europe: A historical introduction. In: Gherardi F, Holdich DM (Eds) Crayfish in Europe as alien species. Balkema Publishers (Rotterdam), 13-22. https://doi.org/10.1201/9781315140469-2

Hoffmann AA, Sgrò CM, Kristensen TN (2017) Revisiting adaptive potential, population size, and conservation. Trends in Ecology & Evolution 32(7): 506–517. https://doi.org/10.1016/j.tree.2017.03.012

Hogger JB (1986) Aspects of the introduction of "signal crayfish", *Pacifastacus leniusculus* (Dana), into the southern United Kingdom. 1. Growth and survival. Aquaculture (Amsterdam, Netherlands) 58(1–2): 27–44. https://doi.org/10.1016/0044-8486(86)90154-7

Holdich DM, Gydemo R, Rogers WD (1999) A review of possible methods for controlling nuisance populations of alien crayfish. In: Gherardi F, Holdich DM (Eds) Crayfish in Europe as alien species. Balkema Publishers (Rotterdam): 245–270. https://doi.org/10.1201/9781315140469-16

Holdich DM, Reynolds JD, Souty-Grosset C, Sibley PJ (2009) A review of the ever-increasing threat to European crayfish from non- indigenous crayfsh species. Knowledge and Management of Aquatic Ecosystems 11(394–395): 394–395. https://doi.org/10.1051/kmae/2009025

Hudina S, Faller M, Lucić A, Klobučar G, Maguire I (2009) Distribution and dispersal of two invasive crayfish species in the Drava River basin, Croatia. Knowledge and Management of Aquatic Ecosystems 9(394–395): 394–395. https://doi.org/10.1051/kmae/2009023

Hudina S, Kutleša P, Trgovčić K, Duplić A (2017) Dynamics of range expansion of the signal crayfish (*Pacifastacus leniusculus*) in a recently invaded region in Croatia. Aquatic Invasions 12(1): 67–75. https://doi.org/10.3391/ai.2017.12.1.07

IUCN (2024) The IUCN Red List of Threatened Species. Version 2024-2. https://www.iucnredlist.org [Accessed on 14 January 2025]

Kirjavainen J, Westman K (1999) Natural history and development of the introduced signal crayfish, *Pacifastacus leniusculus*, in a small, isolated Finnish lake, from 1968 to 1993. Aquatic Living Resources 12(6): 387–401. https://doi.org/10.1016/S0990-7440(99)00110-2

Kouba A, Petrusek A, Kozák P (2014) Continental-wide distribution of crayfish species in Europe: update and maps. Knowledge and Management of Aquatic Ecosystems 413: 05. https://doi.org/10.1051/kmae/2014007

Lodge DM, Taylor CA, Holdich DM, Skurdal J (2000) Nonindigenous crayfishes threaten North American freshwater biodiversity: Lessons from Europe. Fisheries 25(8): 7–20. https://doi.org/10.1577/1548-8446(2000)025<0007:NCTNAF>2.0.CO;2

Lorenzoni M, Ghetti L, Carosi A, Dolciami R (2010) La fauna ittica e i corsi d'acqua dell'Umbria. Sintesi delle Carte Ittiche regionali dal 1986 al 2009. Petruzzi Editore (Perugia, Italy), 1–288.

Lorenzoni M, Croce M, Lorenzoni F, Tagliaferri G, Joubert A, Ricci S, Carosi A (2023) Il gambero della California *Pacifastacus leniusculus* (Dana, 1852) nel Fiume Clitunno (Italia centrale): Monitoraggio e piano di eradicazione. Italian Journal of Freshwater Ichthyology 9(1): 107–124.

Manfrin C, Souty-Grosset C, Anastácio PM, Reynolds J, Giulianini PG (2019) Detection and control of invasive freshwater crayfish: From traditional to innovative methods. Diversity 11(1): 5. https://doi.org/10.3390/d11010005

Marques M, Banha F, Águas M, Anastácio P (2015) Environmental cues during overland dispersal by three freshwater invaders: *Eriocheir sinensis*, *Pacifastacus leniusculus*, and *Procambarus clarkii* (Crustacea, Decapoda). Hydrobiologia 742(1): 81–93. https://doi.org/10.1007/s10750-014-1968-4

Matos M, Teixeira A, Nogueira AB, Padilha J, Sousa R (2025) Brown trout as a potential biological control of signal crayfish. ARPHA Preprint 6: e152051. https://doi.org/10.3897/arphapreprints.e152051

Moorhouse TP, Macdonald DW (2011) The effect of manual removal on movement distances in populations of signal crayfish (*Pacifastacus leniusculus*). Freshwater Biology 56(11): 2370–2377. https://doi.org/10.1111/j.1365-2427.2011.02659.x

Morpurgo M, Aquiloni L, Bertocchi S, Brusconi S, Tricarico E, Gherardi F (2010) Distribuzione dei gamberi d'acqua dolce in Italia. Studi Trentini di Scienze Naturali 87: 125–132.

Pascual R, Piana L, Bhat SU, Castro PF, Corbera J, Cummings D, Delgado C, Eades E, Fensham RJ, Fernández-Martínez M, Ferreira V, Filippini M, García G, Gargini A, Hopper SD, Knapp L, Lewis ID, Peñuelas J, Preece C, Resh VH, Romero E, Samraoui B, Samraoui F, Segadelli S, Skoulikidis NT, Solak CN, Solé J, Villholth KG, Wani HK, Cantonati M, Stevens LE (2024) The Cultural Ecohydrogeology of Mediterranean-Climate Springs: A Global Review with Case Studies. Environments (Basel, Switzerland) 11(6): 110. https://doi.org/10.3390/environments11060110

Pauly D, Munro JL (1984) Once more on comparison of growth in fish and invertebrates. ICLARM Fishbyte 2(1): 21–22.

Préau C, Nadeau I, Sellier Y, Isselin-Nondedeu F, Bertrand R, Collas M, Capinha C, Grandjean F (2020) Niche modelling to guide conservation actions in France for the endangered crayfish *Austropotamobius pallipes* in relation to the invasive *Pacifastacus leniusculus*. Freshwater biology 65(2): 304–315. https://doi.org/10.1111/fwb.13422

Procopio J (2020) *Pacifastacus leniusculus* (Dana, 1852): U.S. Geological Survey, Nonindigenous Aquatic Species Database. https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=200

Rebrina F, Skejo J, Lucić A, Hudina S (2015) Trait variability of the signal crayfish (*Pacifastacus leniusculus*) in a recently invaded region reflects potential benefits and trade-offs during dispersal. Aquatic Invasions 10(1): 41–50. https://doi.org/10.3391/ai.2015.10.1.04

Reid AJ, Carlson AK, Creed IF, Eliason EJ, Gell PA, Johnson PT, Kidd KA, MacCormack TJ, Olden JD, Ormerod SJ, Smol JP, Taylor WW, Tockner K, Vermaire JC, Dudgeon D, Cooke SJ (2019) Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews of the Cambridge Philosophical Society 94(3): 849–873. https://doi.org/10.1111/brv.12480

Ricciardi A, MacIsaac HJ (2011) Impacts of biological invasions on freshwater ecosystems. In: Richardson DM (Ed.) Fifty years of invasion ecology: the legacy of Charles Elton. Blackwell Publishing Ltd (Oxford) 1: 211–224. https://doi.org/10.1002/9781444329988.ch16

Richards KJ (1983) The introduction of the signal crayfish into the United Kingdom and its development as a farm crop. Freshwater Crayfish 5: 557–563. https://doi.org/10.5869/fc.1983.v5.557

Ricker WE (1975) Computation and interpretation of biological statistics of fish populations. J. Bulletin of Fisheries Research Board of Canada 191: 1–382.

Rondinini C, Battistoni A, Teofili C (2022) Lista Rossa IUCN dei vertebrati italiani 2022 Comitato Italiano IUCN e Ministero dell'Ambiente e della Sicurezza Energetica (Roma), 1–57.

Shimizu SJ, Goldman CR (1983) *Pacifastucus leniusculus* (Dana) production in the Sacramento River. Freshwater Crayfish 5: 210–228.

Simberloff D (2009) The role of propagule pressure in biological invasions. Annual Review of Ecology, Evolution, and Systematics 40(1): 81–102. https://doi.org/10.1146/annurev.ecolsys.110308.120304

Sokal RR, Rohlf FL (1987) Introduction to biostatistics. Freeman (New York).

Soto I, Ahmed DA, Beidas A, Oficialdegui FJ, Tricarico E, Angeler DG, Amatulli G, Briski E, Datry T, Dohet A, Domisch S, England J, Feio MJ, Forcellini M, Johnson RK, Jones JI, Larrañaga A, L'Hoste L, Murphy JF, Schäfer RB, Shen LQ, Kouba A, Haubrock PJ (2023) Long-term trends in crayfish invasions across European rivers. The Science of the Total Environment 867: 161537. https://doi.org/10.1016/j.scitotenv.2023.161537

Souty-Grosset C, Holdic DM, Noë PY (2006) Atlas of crayfish in Europe. Muséum National d'Histoire Naturelle (Paris), 1–187.

Souty-Grosset C, Anastácio PM, Aquiloni L, Banha F, Choquer J, Chucholl C, Tricarico E (2016) The red swamp crayfish *Procambarus clarkii* in Europe: Impacts on aquatic ecosystems and human well-being. Limnologica 58: 78–93. https://doi.org/10.1016/j.limno.2016.03.003

Tricarico E, Ghia D, Fea G (2021) Piano di gestione nazionale del gambero della California (*Pacifastacus leniusculus*). Ministero della Transizione Ecologica (Roma), 1–25.

Tucker EA, Tucker ME (2019) Crayfish gastroliths. Geology Today 35(1): 26–28. https://doi.org/10.1111/gto.12254

Twardochleb LA, Olden JD, Larson ER (2013) A global meta-analysis of the ecological impacts of nonnative crayfish. Freshwater Science 32(4): 1367–1382. https://doi.org/10.1899/12-203.1

Vaeßen S, Hollert H (2015) Impacts of the North American signal crayfish (*Pacifastacus leniusculus*) on European ecosystems. Environmental Sciences Europe 27(1): 1–6. https://doi.org/10.1186/s12302-015-0065-2

von Bertalanffy L (1938) A quantitative theory of organic growth. Human Biology 10: 181–243.

Wacker A, Harzsch S (2021) Crustaceans in a changing world. Zoology : Analysis of Complex Systems, ZACS 146: 125921. https://doi.org/10.1016/j.zool.2021.125921